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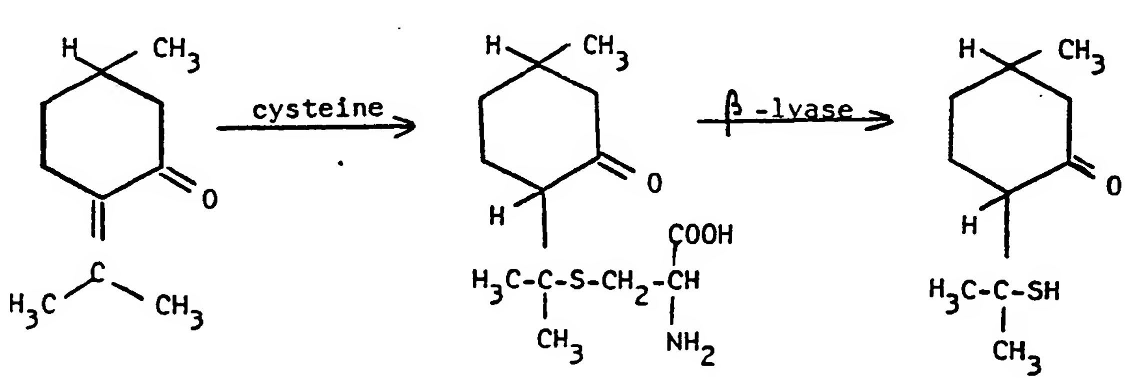
(54) Method for preparing thiol compounds.

(57) Method for preparing thiol compounds by coupling cysteine having the formula $HS\text{-}CH_2\text{-}CH(NH_2)COOH$ via an -S-bridge to a hydrocarbon compound and subsequently reacting the cysteine conjugate obtained with β-lyase to form the relevant thiol compounds. For instance it is possible to prepare the flavour p-mentha-8-thiol-3-one starting from pulegone as illustrated in the diagram below:



EP 0 277 688 A2

Method for preparing thiol compounds.

The invention relates to a method for preparing thiol compounds.

In Pesticide Biochemistry and Physiology 14, pages 50-61 (1980), the in vitro metabolism of pentachloronitrobenzene (PCNB) into pentachloromethylthiobenzene (PCTA) by means of an enzyme system obtained from onions is described. More particularly, this reference relates to the in vitro preparation of PCTA from PCNB at a pH of 7.9 by means of an enzyme system which contains dithiothreitol, glutathione and S-adenosylmethionine. Said enzyme system was prepared from onion roots by ammonium sulphate fractionation and differential centrifugation. The enzyme system contained glutathione-S transferase activity with PCNB, C-S-lyase activity (also termed β-lyase activity) with S-(pentachlorophenyl)cysteine, S-adenozylmethionine-methyl transferase activity with pentachlorothiophenol (PCTP) and probably a few other peptidase activities. The yield of the thiol compound concerned, namely pentachlorothiophenol (PCTP) is, however, negligible in this known method compared with the yield of PCTA (see page 55, right-hand column, lines 10-13 from bottom) so that this method is considered unsuitable for preparing thiol compounds.

In Journal of Biological Chemistry, vol. 253, 24, pages 8854-8859 (1978), the cysteine conjugate β-lyase in rat liver is described. This enzyme catalysing cleavage of the thioether linkage in cysteine conjugates has been purified about 500-fold from rat liver cytosol. However, according to the Chapter "Assay Methods" (page 8855) the obtained thiol compounds were directly methylated whereafter the methylated derivatives were identified by spectroscopy methods.

A method defined in the introduction has now been found which is characterized in that cysteine is coupled via an -S-bridge to a hydrocarbon compound and subsequently the cysteine conjugate obtained is reacted with a β-lyase to form the thiol compound(s) concerned and also NH_3 en CH_3-CO-COOH.

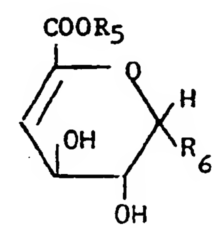
From the above it may be inferred that the method according to the invention can be subdivided into two steps:

a) the preparation of the cysteine conjugate; and

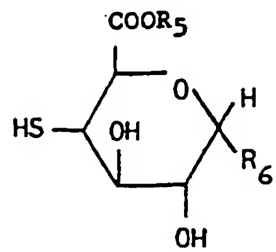
b) the splitting of said cysteine conjugate into, inter alia, the thiol compound(s) concerned.

The preparation of the cysteine conjugate may be carried out, for example, by an addition or substitution reaction. More particularly, the addition reaction of cysteine can be carried out with a compound having the formula $(R_1)(R_2)C=C(R_3)$-CO-R_4 in which the symbols R_1-R_4 represent a hydrogen atom or an optionally saturated and/or heterogeneous hydrocarbon group or, together with the carbon atom to which the symbols are bonded, form one or two, optionally saturated and/or heterogeneous ring systems. For example, the symbols R_1-R_4 represent a hydrogen atom, an alkyl group containing 1-5 carbon atoms, an alkenylene group containing 2-5 carbon atoms, a cycloalkyl or cycloalkenyl group containing 5-10 carbon atoms or an aryl group containing 6-10 carbon atoms, which abovementioned groups may be substituted by halogen atoms and/or one or more groups containing carbon, nitrogen, sulphur, oxygen and/or halogen atoms. Preferably, the symbols R_2 and R_3 represent a hydrogen atom or an alkyl group containing 1-3 carbon atoms and R_4 an optionally heterogeneous hydrocarbon group bonded via an -O-bridge.

For example, unsaturated sugars having the formula



in which the symbol R_5 represents a hydrogen atom, an alkyl group containing 1-24 carbon atoms or an alkaline ion and R_6 represents a group consisting of 1-7 monosaccharides selected from the group consisting of glucose, mannose, galactose, arabinose, fucose, xylose, rhamnose, uronic acids and derivatives thereof like the acetates, pyruvates, amines and sulphates are also suitable as starting material for the addition reaction of cysteine. Preferably R_6 represents a glucose-rhamnose-glucose group. The obtained cysteine-conjugates are simply convertable to compounds with the formula



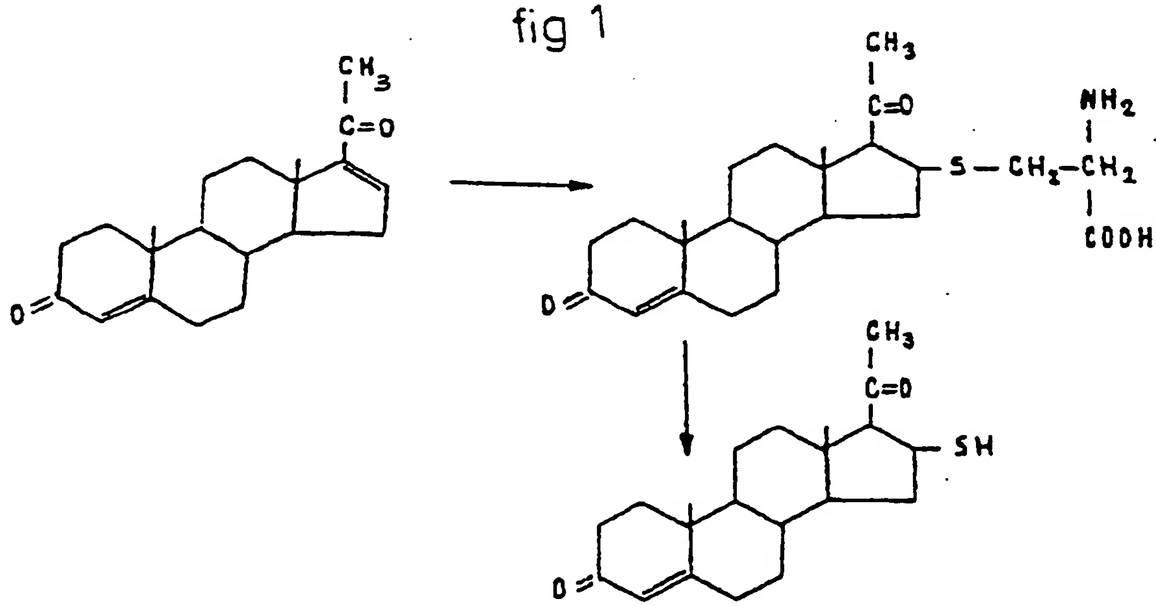
having flavouring properties.

The above addition reactions can be carried out in a purely chemical manner but also in a biochemical manner under the unfluence of an enzyme such as esterase or lipase.

The preparation of a cysteine conjugate by means of a substitution reaction can be carried out by means of nucleophilic substitution of glutathione in the presence of glutathione transferase, it being possible for Cl, NO_2 and H to appear as the group to be replaced. The glutathione conjugate is converted enzymatically into the cysteine conjugate (the glutathione conjugate is converted by means of a carboxy peptidase into the γ-glutamylcysteine conjugate which is in turn converted into the cysteine conjugate under the influence of γ-glutamyl transpeptidase). However, such a synthesis route is not as yet being used advantageously owing to the additional processing steps.

Many types of cysteine conjugates are known as such from the prior art. For example, the preparation of such cysteine conjugate is known from Applied and Environmental Microbiology, May 1985, pages 1146-1153. In this reference, 16-dehydroprogesterone, in particular, is converted with L-cysteine in a non-enzymatic manner into 16-S-cysteinylprogesterone. Said cysteinyl compound can be converted in the presence of β-lyase into 16-mercaptoprogesterone by means of the second stage of the method according to the invention. The diagram below illustrates the synthesis route described above:

fig 1



The thiolsteriod shown above has specific pharmacological properties.

The formation of cysteine conjugates of 3-(3,4-dihydroxyphenyl)analine is reported in Biochimica et Biophysica Acta 672 (1981), pages 151-157. As indicated on page 155 of this reference, polyconjugates can also be obtained in addition to some monoconjugates. These singly or multiply conjugated compounds can also be converted by means of the β-lyase to be used according to the invention into the corresponding mono-or polythiol derivatives.

Reference may be made to the following additional references relating to specific cysteine conjugates or derivatives derived therefrom:

1) J.Chem.Soc.Chem.Commun.1986, pages 1331-1333;
2) Journal of Food Science, vol. 51, no. 5, 1986, pages 1191-1194;
3) Planta (1986) 169: 208-215; and
4) Carbohydrate Research 142 (1985), pages 93-105.

The cysteine used in the method according to the invention has the formula $HS\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$. In view of the spectrum of activity of the β-lyase to be used in the method according to the invention, L-cysteine is used.

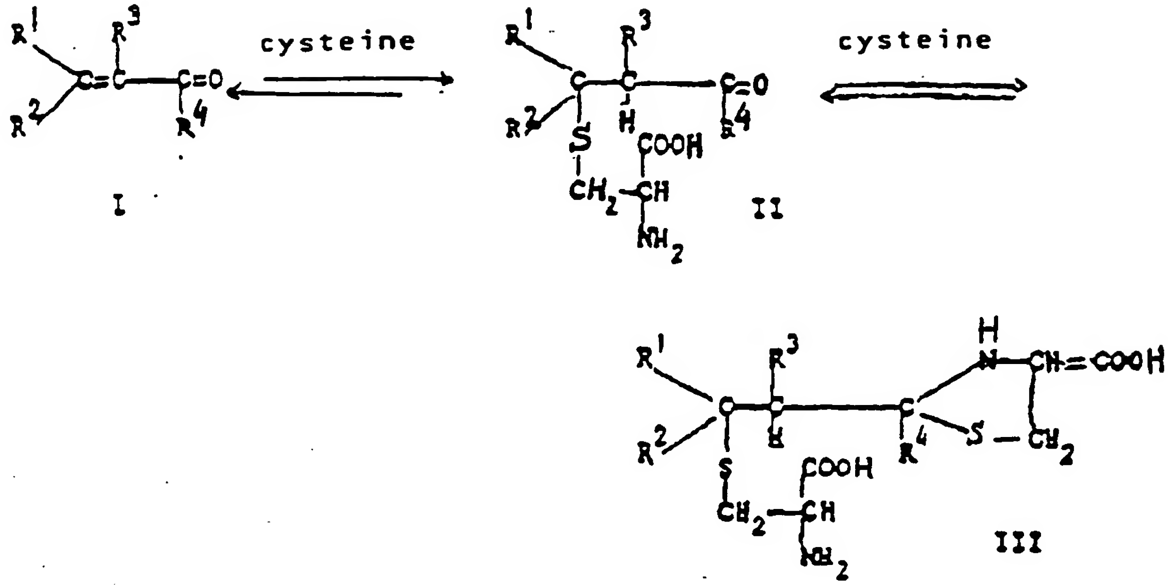
The β-lyase (also termed C-S-lyase or cysteine conjugate β-lyase) to be used in the method according to the invention is an enzyme dependent on a pyridoxal 5-phosphate (vitamin B6). In addition to being present in a large number of intestinal bacteria (in 24 out of the 43 intestinal bacteria investigated), the β-lyase is also present in some vegetable and animal cells (Larsen G.L., "Distribution of cysteine conjugate β-lyase in gathrointestinal bacteria and the environment, Xenobiotica 15, 199-209 (1985)). The bacterial β-lyases are able to convert a wide spectrum of substrates, in particular both S-alkyl-and S-arylcysteine conjugates, whereas the spectrum of activity of β-lyase of vegetable or animal origin is limited. Measured with the cysteine-propachlor conjugate (an S-alkylcysteine conjugate), the β-lyase originating from the anaerobic intestinal bacterium Eubacterium limosum is the most active enzyme and has the lowest substrate specificity (Larsen, loc. cit.). If, however, the conversion of S-(2-benzothiazolyl)cysteine (an S-arylcysteine conjugate) is examined, it emerges that the β-lyase from an anaerobic Fusobacterium species has virtually an identical activity. β-lyase from F.necrophorum and E. limosum differ not only in substrate specificity, but also in size, namely 228 kd and 75 kd (2x38 kd) and also in stability. The enzyme from F.necrophorum requires pyridoxal 5-phosphate for stability but is then also more stable to heat. β-lyase from E.limosum and F.varium exhibit no activity with D-cysteine conjugates and have, in general, a lower activity for S-alkylcysteine conjugates than for S-arylcysteine conjugates.

The isolation of β-lyase from both E.limosum and F.varium does not have to be carried out under anaerobic conditions. This indicates that the enzyme is not sensitive to oxygen. IT also emerges from the isolation method that the enzyme is located in the cell. The second step described above of the method according to the invention can therefore be carried out with purified/extracted β-lyase or, if the substrates are absorbed by the bacterial cells and are converted therein, with the respective bacteria themselves.

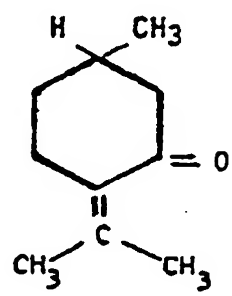
The method according to the invention results in many types of thiol compounds with divergent applications. Examples of substances to be prepared pertain to the field of perfumes and flavouring (p-mentha-8-thiol-3-one, damascone derivative), pharmacological steroid compounds and repellants (Warburganal).

The invention further relates to the purification or separation of α,β-unsaturated and also saturated aldehydes and ketones from, in particular, complex vegetable products using the cysteine conjugates derived therefrom and also to the enrichment associated therewith of the residual substances also present in said products. After separation from the original product by means of, for example, steam distillation, the cysteine conjugates formed are split into the purified aldehyde or ketone and the cysteine. This recovered cysteine can subsequently be employed again in the cysteine conjugate preparation.

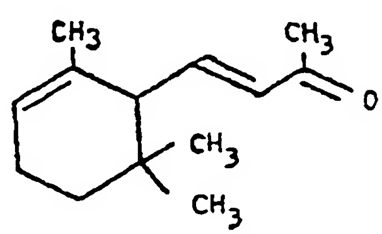
The formation of the cysteine conjugate (II) is shown on the basis of the diagram below for α,β-unsaturated aldehydes and ketones (I) having the formula $(R_1)(R_2)C=C(R_3)\text{-}(0\text{-}R_4)$ in which R_1-R_4 have the meaning stated above; this conjugate formation is often followed by the attachment of a cysteine molecule to the carbonyl group of the aldehyde or ketone to form a thiazolidine-4-carboxylic acid derivative (III).



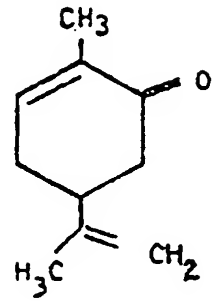
As examples of such α,β-unsaturated aldehydes and ketones, mention may be made of:
- pulegone having the formula



- α-ionone having the formula

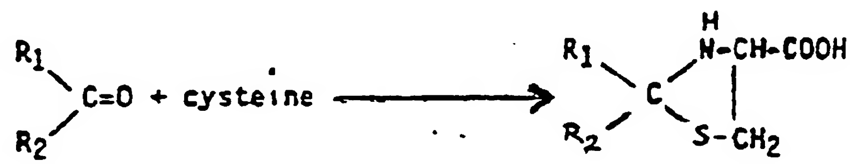


- carvone having the formula

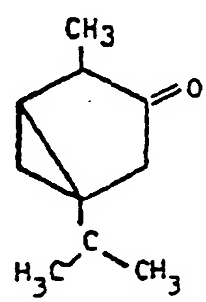


and
- citral having the formula $(CH_3)_2C=CH\text{-}CH_2\text{-}CH_2\text{-}(CH_3)C=CH\text{-}CHO$.

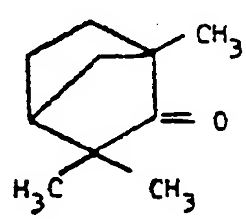
For saturated aldehydes and ketones having the formula $(R_1)(R_2)C=0$ in which R_1 and R_2 have the abovementioned meaning, the formation of the thiazolidine-4-carboxylic acid derivatives derived therefrom may be represented as follows.



As examples of such saturated aldehydes and ketones mention may be made of:
-thujone having the formula



and
- fenchone having the formula



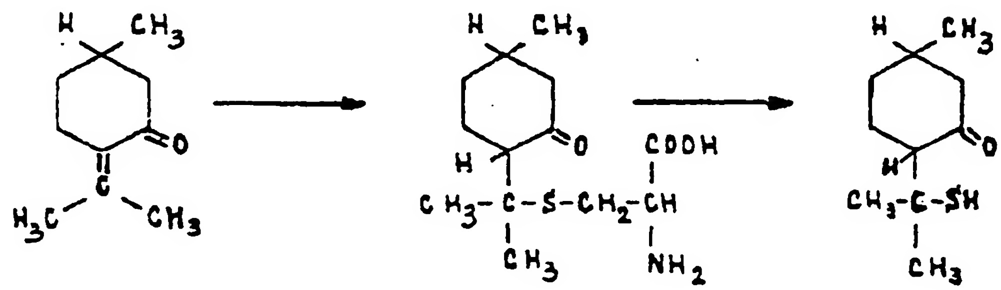
An important advantage of the purification described above lies in the fact that no biologically foreign reagents such as bisulphite (not usable in the case of α,β-unsaturated carbonyl compounds), hydroxylamine, 1-naphthylamine-5-sulphonic acid, hydrazine, thiosemicarbazide etc have to be used. The purification can also be carried out under mild conditions as regards pH and temperature and the cysteine is recovered.

The invention is explained on the basis of the examples below, Examples I and II relating to the thiol

preparation and Example III relating to the puricication method; these examples should not be interpreted as restrictive.

Example I

In this example, the starting point is pulegone, which is converted via S-cysteinyl— pulegone into p-mentha-8-thiol-3-one. This preparation is illustrated in the diagram below.



Stage 1) Preparation of S-cysteinyl— pulegone.

12.2 g of L-cysteine (0.1 mol) (high punty analytical grade supplied by Fluka A.G.), 16.3 ml of pulegone (0.1 mol) and 2.0 g of $KHCO_3$ (0.02 mol) were stirred for 22 hours in 100 ml of H_2O at room temperature. The yoghurt-like mixture, which was no longer stirrable, was then allowed to stand for 3 days. The product obtained was then filtered off by suction and washed respectively with 100 and 2 x 50 ml of H_2O. After drying over $CaCl_2$ in vacuo, the product was washed with acetone. The yield was 17.9 g. Appendix 1 shows the 90 MHz H-NMR spectrum of the product obtained.

More particularly, an elementary analysis of the product purified by thin-layer chromatography clearly indicates a 1:1 reaction product.

Elementary analysis (carried out in duplicate)

Found:		Calculated (substance + 1/2 mol of H_2O):
%C:	54.76	55.29
%H:	8.36	8.57
%N:	5.01	4.96
%O:	19.60	19.83
%S:	11.13	11.35

Stage 2) Splitting of the S-cysteinyl— pulegone

The organism used in this stage is Eubacterium limosum having the ATCC no. 10825. Said organism was cultured under anaerobic conditions at 37°C on a P-medium which had the composition below:

Composition of P-medium:Casein peptone (Difco) 10 g/l
Beef extract (Difco) 3 g/l
Yeast extract (Difco) 3 g/l
Glucose (Merck) 2 g/l
Tween 80 (Serva) 1 g/l
Cysteine-HCl (Fluka) 0.5 g/l
Resazurin (Serva) 0.25 g/l
Salt solution (analytical grade) 40 ml/l
Final pH: 7.2

The salt solution consisted of:$CaCl_2$ 0.2 g/l
$MgSO_4.7H_2O$ 0.2 g/l
K_2HPO_4 1.0 g/l
KH_2PO_4 1.0 g/l
$NaHCO_3$ 10.0 g/l
NaCl 2.0 g/l

The cell material for producing β-lyase was obtained by culturing E.limosum (3% inoculation) on the abovementioned P-medium in serum bottles having a capacity of 300 ml. By filling the bottle with P-medium to a few centimetres below the rim, the medium became sufficiently low in oxygen as a result of sterilization to make growth of E.limosum possible. After an incubation time of 1 day at 37°C, the cells were harvested by centrifuging them at 50,000 x g for 20 minutes. The cells were subsequently washed twice with a buffer having a pH of 7 which contained 50 mM of phosphate and 50 mM of pyridoxal-HCl. The pellet (approx. 1 g wet weight from 300 ml) was taken up in 10 ml of buffer.

S-cysteinyl— pulegone (0.3 g/l = 1.1 mM) was converted in the buffer with the concentrated cell suspension of E.limosum described above (final concentration: 1.6 mg dry weight/ml). The reaction was carried out for 1 hour at 30°C and was terminated by centrifuging the reaction mixture for 5 minutes at 11,000 x g.

As a control, two tests were carried out:

a) As a control, boiled cells (denatured enzymes) were used in the test described above.

b) In order to be able to assess whether the SH product (p-mentha-8-thiol-3-one) had converted by the S-methyl transferase into the S-methyl product (p-mentha-8-thiomethyl-3-one), the cells were also incubated with p-mentha-8-thiol-3-one.

The results of gas chromatography analysis of this example (samples no. 1) and the two control tests (samples 2 and 3) are shown in Appendix 2.

To carry out the abovementioned gas chromatographic analysis, 1 part of chloroform ($CHCl_3$) was mixed with 1 part of the reaction mixture obtained. 1 µl of this extract was injected into a gas chromatograph having a 20 M carbowax column, (1.3 m RVS, column temperature: 145°C, injection port and TCD temperature: 160°C).

Example II

The method according to Example I was repeated, but with the difference that, instead of being carried out on a 1 ml scale, the test was carried out on a 10 ml scale. In this test, the cells were used in a double concentration, viz. 3.2 mg dry weight/ml and the incubation was carried out for 3 hours at 37°C. For a gas chromatographic analysis, a sample (sample B) was taken from this in the following manner.

One part of dichloromethane (CH_2Cl_2) was mixed with 4 parts of the reaction mixture. 0.4 µl of this extract was injected into a Varian gas chromatograph in which a 10 % FFAP-chromosorb was provided in a WAW column (2m RVS, i.d. 1/8") (column temperature: 160°C; injection port and FID temperature: 180°C).

As a comparison, in addition to the gas chromatogram of sample B shown in Appendix 3 as a control, the gas chromatograms of a) p-mentha-8-thiol-3-one, b) p-metha-8-thiomethyl-3-one, c) pulegone, and d) S-cysteinylpulegone were recorded without cells being used at the same time.

It follows from the chromatograms shown in Appendix 3, inter ali, that no detectable p-methan-8-thiomethyl-3-one is formed (compare 3b with 3e). The pulegone peak in Figures 3d and 3e (retention time 2.7 min.) may be explained by the fact that some of the S-cysteinyl— pulegone dissolves in the extraction

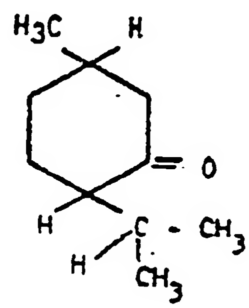
agent and is decomposed in the gas chromatograph (160°C).

The chromatogram of chemically synthesized p-mentha-8-thiol-3-one (Fig. 3a) reveals an isomer ratio of approximately 2:1. The biologically prepared p-mentha-8-thiol-3-one (Fig. 3e) has a completely different ratio of the two isomers which is approximately 9:1.

Example III

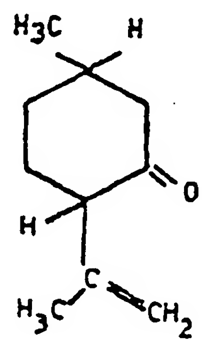
As the starting product, a pulegone product of vegetable origin having a pulegone content determined by gas chromatography of 87.7 % by weight was used. As impurities in such a product, mention is made, inter alia, of

- L-menthone having the formula



and

- isopulegone having the formula



which cannot be separated or can virtually not be separated from pulegone, for example, by means of fractional distillation.

2,5 g of S-cysteinyl-pulegone.1/2 H_2O was prepared in accordance with the manner stated in stage (1) of Example I. This product was subjected to a steam distillation until no further pulegone distilled over. Subsequently, the distillate was extracted with carbon tetrachloride, after which the extract obtained, after drying over sodium sulphate, was evaporated down under vacuum. The yield was 1.3 g of pulegone (96.4 % of the theoretical quantity) which had a purity of 97.9 % as determined by gas chromatography.

Example IV

A commercial cocoa mix is used to prepare two different batches of beverage. The first batch is evaluated without any further addition while p-mentha-8-thiol-3-one prepared according to Example II is added to the second batch in the ratio of 20 µg of said p-mentha-8-thiol-3-one to each kilo of cocoa beverage. The beverage containing p-mentha-8-thiol-3-one has a fuller and richer flavour comparing to the beverage without p-mentha-8-thiol-3-one.

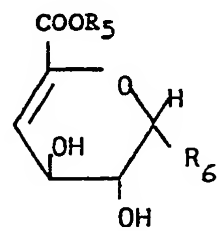
Claims

1. Method for preparing thiol compounds, characterized in that cysteine is coupled via an -S-bridge to a hydrocarbon compound and subsequently the cysteine conjugate obtained is reacted with β-lyase to form one or more thiol compounds.

2. Method according to Claim 1, characterized in that cysteine is coupled by means of addition to a compound having the formula $(R_1)(R_2)C=C(R_3)$-CO-R_4 in which the symbols R_1-R_4 represent a hydrogen atom or an optionally saturated and/or heterogeneous hydrocarbon group or, together with the carbon atoms to which the symbols are bonded, form one or two, optionally saturated and/or heterogeneous ring systems and subsequently the cysteine conjugate obtained is reacted with β-lyase to form the relevant thiol compounds.

3. Method according to Claim 2, characterized in that cysteine is coupled by means of addition to a compound having the formula $(R_1)(R_2)C=C(R_3)$-CO-R_4 in which R_1 has the meaning stated in Claim 2, R_2 and R_3 represent a hydrogen atom or an alkyl group containing 1-3 carbon atoms and R_4 represents an optionally heterogeneous hydrocarbon group bonded via an -O-bridge and subsequently the cysteine conjugate obtained is reacted with β-lyase to form the relevant thiol compounds.

4. Method according to Claim 2, characterized in that cysteine is coupled by means of addition to a compound having the formula



in which the symbol R_5 represents a hydrogen atom, an alkyl group containing 1-24 carbon atoms or an alkaline ion and R_6 represents a group consisting of 1-7 monosaccharides selected from the group consisting of glucose, mannose, galactose, arabinose, fucose, xylose, rhamnose, uronic acids as well as the acetates, pyruvates, amines and sulfates derived therefrom and subsequently the cysteine conjugate obtained is reacted with β-lyase to form the relevant thiol compounds.

5. Method according to Claims 2, 3 or 4, characterized in that the addition is carried out in a biological manner under the influence of an enzyme such as esterase or lipase.

6. Method according to Claim 1, characterized in that cysteine in the form of glutathione is coupled to a hydrocarbon compound by means of nucleophilic substitution under the influence of glutathione-transferase.

7. Method according to one or more of the Claims 1-6, characterized in that the cysteine conjugate is split by means of bacterial β-lyase.

8. Method according to Claim 7, characterized in that β-lyase from Eubacterium limosum is used.

9. Method accoding to Claims 1, 7 or 8, characterized in that β-lyase is used in the form of bacterial cells.

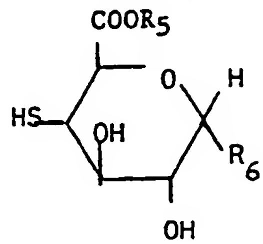
10. Method according to one or more of the Claims 1-9, characterized in that the flavouring p-mentha-8-thiol-3 one is prepared starting from pulegone.

11. Method according to one or more of the Claims 1-9, characterized in that the compound 16-mercapto-progesterone is prepared starting from 16-dehydroprogesterone.

12. Cysteine conjugates obtained in the method according to one or more of the Claims 1-11, with the proviso that S-(pentachlorophenyl)cysteine, 16-S-cysteinylprogesterone and the cysteinyl derivatives of 3-(3,4-dihydroxyphenyl)alanine, 2,4-dinitrobenzene and p-bromobenzene are exclused.

13. Flavour composition comprising an effective flavouring amount of p-mentha-8-thiol-3-one, together with customary ingredients.

14. Flavour composition comprising an effective flavouring amount of one or more flavouring compounds of the formule

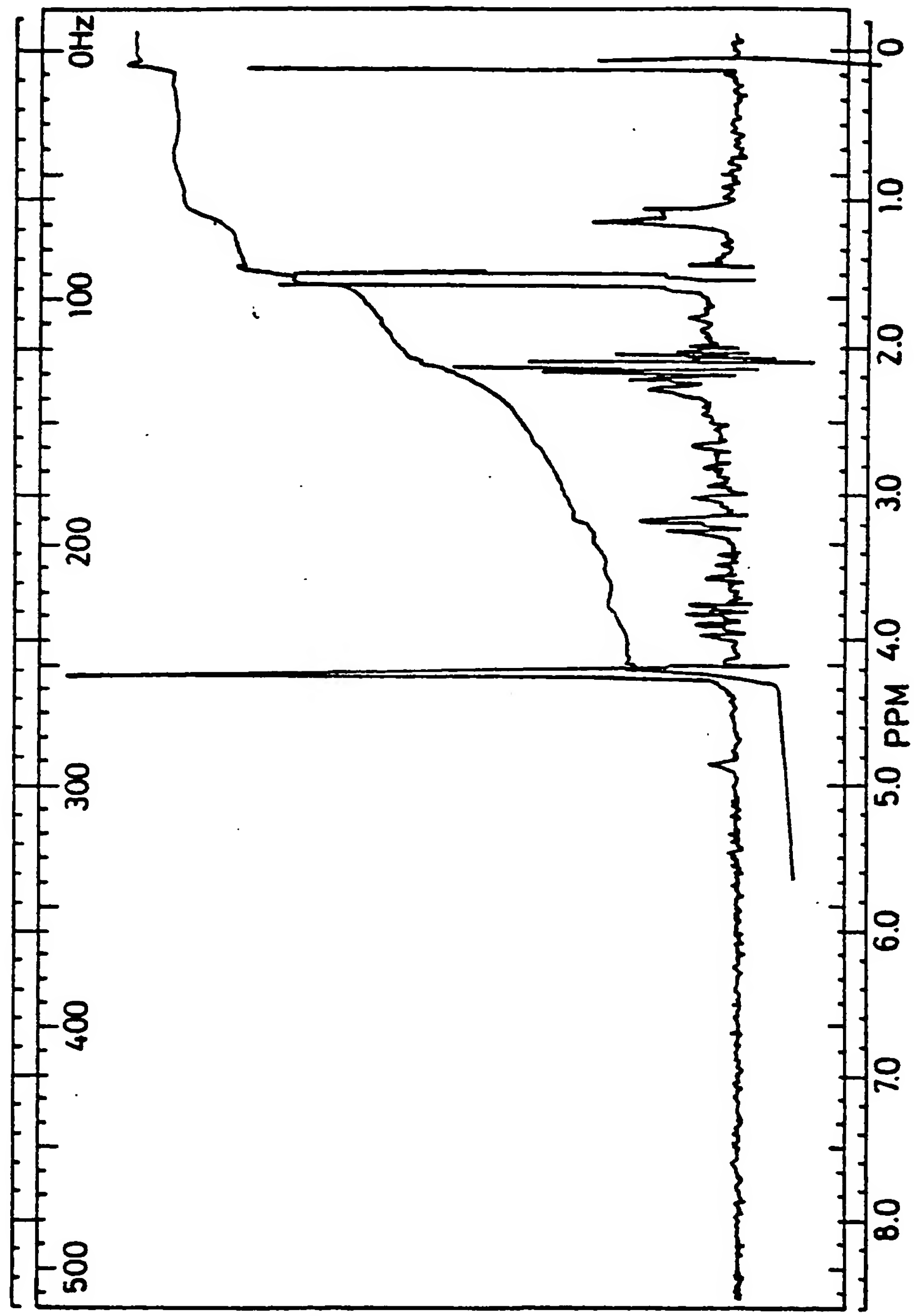


in which the symbol R_5 represents an hydrogen atom, an alkyl group containing 1-24 carbon atoms or an alkaline ion and R_6 represents a group consisting of 1-7 monosaccharides selected from the group consisting of glucose, mannose, galactose, arabinose, fucose, xylose, rhamnose, uronic acids as well as the acetates, pyruvates, amines and sulfates derived therefrom, together with customary ingredients.

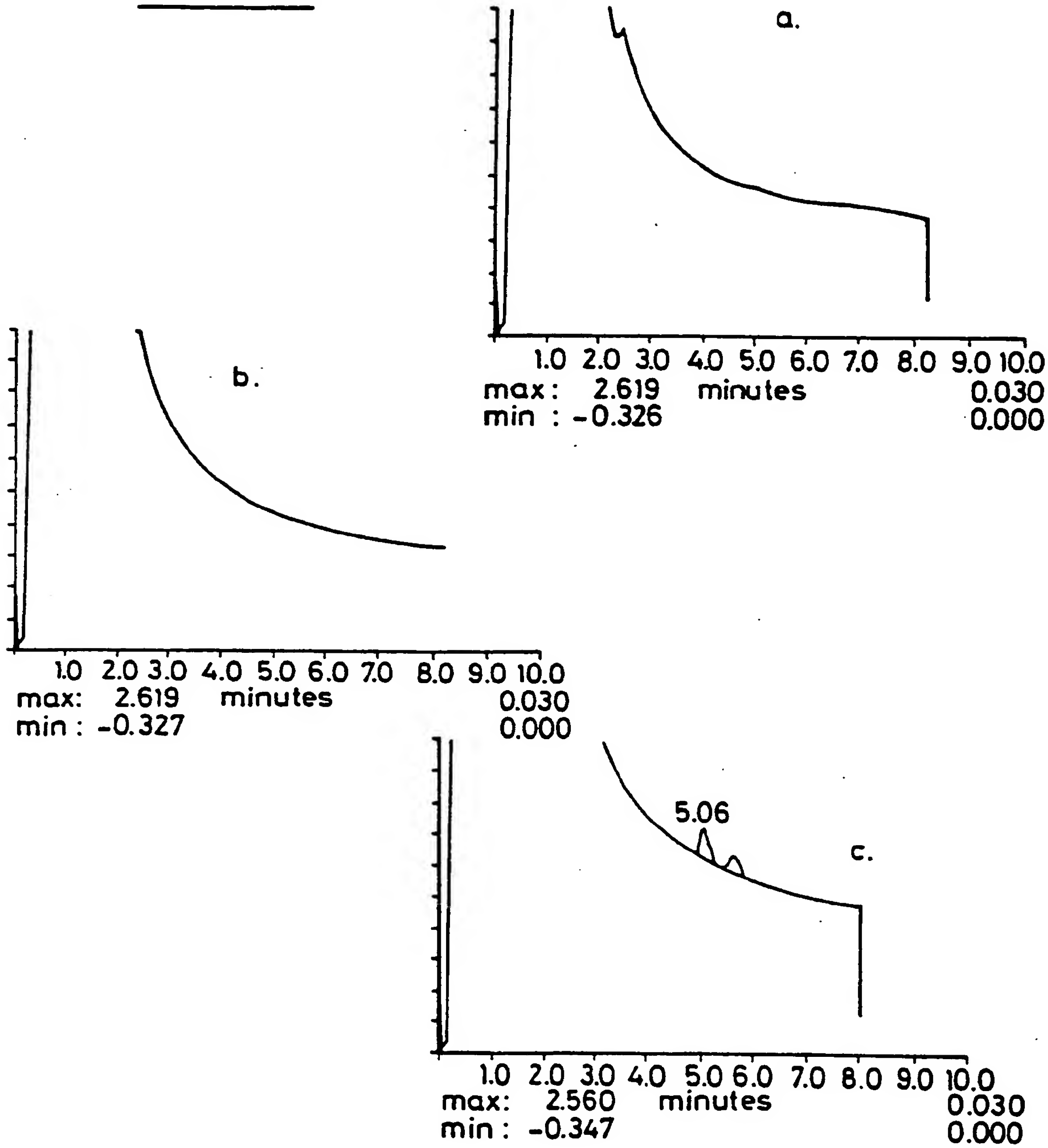
15. Method for purifying or separating aldehydes and ketones, in particular α,β-unsaturated aldehydes and ketones, from products containing such carbonyl compounds, characterized in that cysteine is bonded to the respective aldehyde or ketone via an -S-bridge, and cysteine conjugate obtained is isolated from the reaction mixture and subsequently, the conjugate obtained is split into cysteine and the respective aldehyde or ketone.

16. Method according to Claim 15, characterized in that contaminated pulegone is converted into S-cysteinyl-pulegone with cysteine and the isolated conjugate obtained is subjected to a steam distillation to obtain purified pulegone.

APPENDIX 1



APPENDIX 2

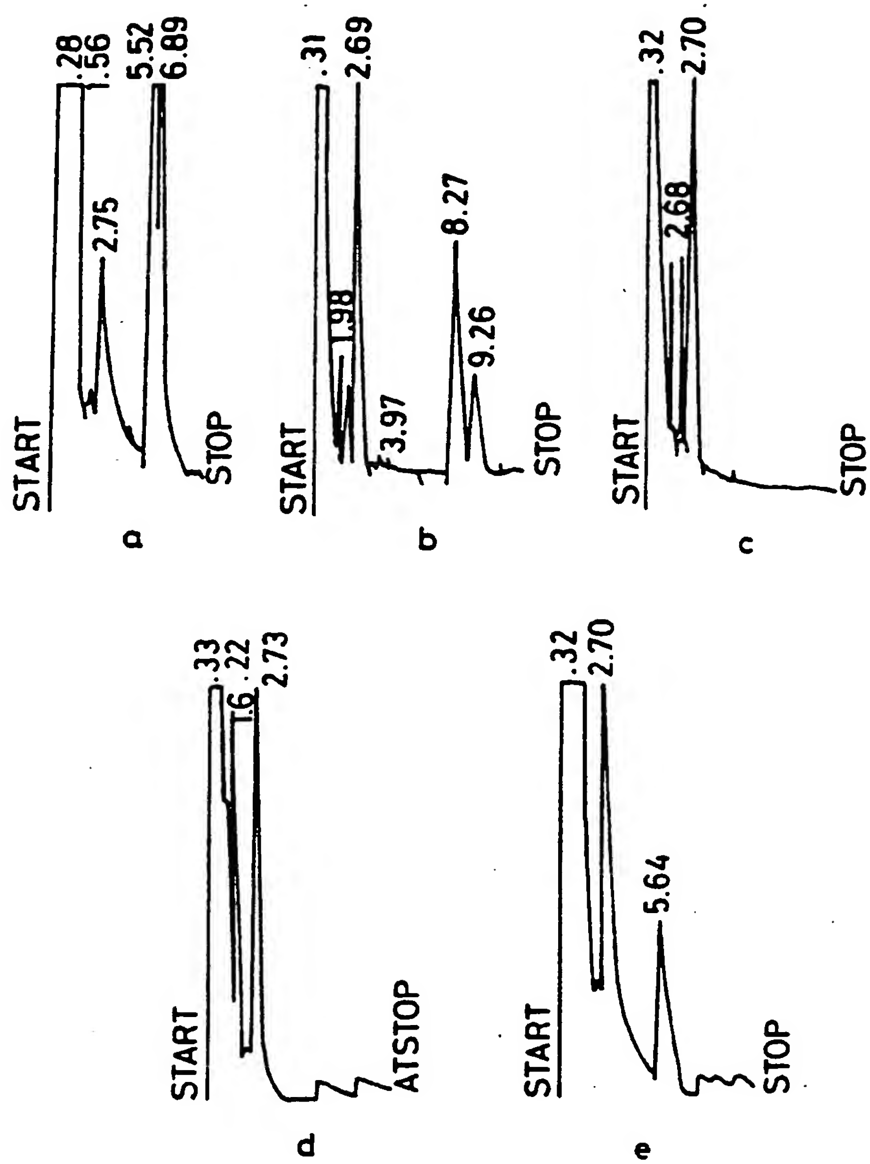


Chromatograms of the samples of Example 2

a. sample no. 1
b. sample no. 2
c. sample no. 3

Note : The samples have been extracted with CHCl (1 to 1)
1 μl of this extract has been analysed by gas chromatography.

APPENDIX 3



Chromatograms of some extracts of example 2

a 0.02% TF in buffer (recorder : 1 mV F.S.)
b 0.02% TFM in buffer (recorder : 2 mV F.S.)
c 0.02% pulegone in buffer (recorder : 2 mV F.S.)
d 0.03% S-cysteinyl-pulegone in buffer (recorder : 1/2 mV F.S.)
e Sample B (recorder : 1/2 mV F.S.)

Note : The samples have been extracted with CH_2Cl_2 (4 to 1).
0.4 μl of this extract was analysed by gas chromatography.

F.S. = full scale

TF= p-mentha-8-thiol-3 one
TFM = p-mentha-8-thiomethyl-3-one

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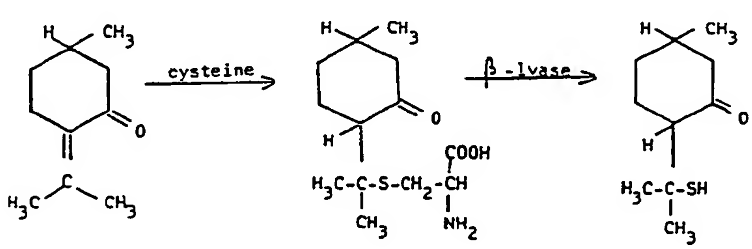
(54) **Method for preparing thiol compounds.**

(57) Method for preparing thiol compounds by coupling cysteine having the formula $HS-CH_2-CH(NH_2)COOH$ via an -S-bridge to a hydrocarbon compound and subsequently reacting the cysteine conjugate obtained with β-lyase to form the relevant thiol compounds. For instance it is possible to prepare the flavour p-mentha-8-thiol-3-one starting from pulegone as illustrated in the diagram below:



EP 0 277 688 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application number
EP 88 20 0141

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 253, no. 24, 25th December 1978, pages 8854-8859, US; M. TATEISHI et al.: "Cysteine conjugate beta-lyase in rat liver. A novel enzyme catalyzing formation of thiol-containing metabolites of drugs" * Page 8858 *	1	C 12 P 11/00 C 12 P 33/00 A 23 L 1/226 C 07 C 45/78
X	MICROECOLOGY AND THERAPY, vol. 15, 1985, pages 261-266, Institut für Mikroökologie, Herborn-Dill, DE; G.L. LARSEN et al.: "Microfloral cysteine conjugate beta-lyase: an enzyme responsible for formation of xenobiotic thiols in the gastro-intestinal tract" * Whole document *	1,7,8	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C 12 P 11/00
C 07 B 45/00
C 07 C 148/00
C 07 C 149/00
C 12 P 33/00
C 07 J 31/00

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
The Hague	13-04-1988	VAN GEYT

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

X LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

1. Claims 1-12: method for preparing thiols
2. Claim 13: flavour composition comprising p-mentha-8-thiol-3-one
3. Claim 14: flavour composition comprising saccharide derivatives
4. Claims 15,16: method for purifying aldehydes and ketones

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,

namely claims: 1-12